FURTHER STUDIES ON THE LIFE CYCLE OF PARAMECIUM.

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I. Introduction.

The life cycle of infusoria has been the subject of numerous investigations since Ehrenberg suggested on *a priori* grounds that the protozoa are so simply organized that they are not subject to natural death, and Dujardin opposed the view and maintained that the life history of infusoria comprises a cyclical change in vitality which terminates in death.

Bütschli ('76), Engelmann ('76), Maupas ('88; '89), Joukowsky ('98), Simpson ('01), Calkins ('02; '04), Woodruff ('05), Popoff ('07) and Gregory ('09) have all advanced evidence tending to show that infusoria when bred under somewhat constant culture conditions pass through a more or less definite physiological cycle. This cycle is characterized by an initial high potential of division which gradually is expended until reproduction finally ceases, and death puts an end to the cycle unless conjugation is permitted or artificial stimuli are employed. Characteristic morphological changes, both cytoplasmic and nuclear, appear in many cases as "senile degeneration" increases.

Enriques ('08) in a recent paper has again opposed the idea of old age and physiological death in protozoa and has contended that the results which support the cyclical character of the infusorian life history have been obtained by faulty culture methods. The conclusion of Enriques is, I believe, somewhat too sweeping, and is based in part on a misunderstanding of the methods by which the most extensive cultures have been con-

ducted. The work on the infusorian life history has clearly shown that many species of infusoria, when bred on a more or less constant culture medium, pass through quite definite cycles. Calkins, Woodruff, and Gregory have shown also that specific changes in the environment at critical times may "rejuvenate" a culture and lengthen its life for long periods. It is demonstrated, I believe, that the life history of the infusorian is cyclical when subjected to a constant environment, and it is also demonstrated that the life history may be lengthened by the timely use of various stimuli.

I have defined a cycle as "a periodic rise and fall in the fission rate, extending over a varying number of rhythms, and ending in the extinction of the race, unless it is 'rejuvenated' by conjugation or *changed environment*."[1] This suggests the idea that it may be possible to eliminate the cyclical character of the division rate by *constantly* subjecting the organisms to a varied environment and the present investigation is devoted to this aspect of the problem. In a former paper[2] I have given an outline of my studies up to May, 1908, on the life history of *Paramecium* when subjected to a varied environment. The present paper presents the data to June 29, 1909.

II. Methods.

A "wild" *Paramecium aurelia* (*caudatum*) was isolated from a laboratory aquarium on May 1, 1907, and placed in about five drops of culture medium on an ordinary glass slide having a central ground concavity. When this organism had divided twice, producing four individuals, each of these were isolated on separate slides to start the four lines, *I–a*, *I–b*, *I–c* and *I–d* which compose this culture (Paramecium I).[3] The culture has been continued by the isolation of an individual from each of these lines almost daily throughout the life of the culture up to the present time (June 29, 1909). A record has been kept of the daily divisions of each line, and the average rate of division of the four lines of the culture and this again averaged for five- ten- and thirty-day

[1] Woodruff ('05).

[2] Woodruff ('08[2]).

[3] For further details in regard to technique see Woodruff ('05).

periods has been plotted (cf. Figs. 1, 2 and 3). Permanent preparations have been preserved at various periods in the life history for the purpose of studying the cytoplasmic and nuclear changes, if present.

The culture was carried on at the Thompson Biological Laboratory of Williams College, Williamstown, Mass., during May and June, 1907; at the Marine Biological Laboratory, Woods Holl, Mass., during July and August, 1907 and 1908; and at the Sheffield Biological Laboratory of Yale University, New Haven, Conn., from September, 1907, to July, 1908, and from September, 1908, to the present time (June, 1909).

During the first nine months of the work the culture medium was made of hay or grass; but, except during certain periods in which the culture was employed as a control for special experiments,[1] the infusion was made with hay from various localities, and different proportions of hay and water were used almost daily. Water from different sources was employed. The temperature of the infusion was always raised to the boiling point. In some cases the infusion was used as soon as it had again attained the room temperature; in others, it was allowed to stand for twenty-four hours before it was employed.

From February, 1908, to the present time, June, 1909, however, a more varied culture medium was employed. *Paramecium* will thrive in nearly any infusion which may be made from materials collected in ponds and swamps, and therefore, in an endeavor to supply as far as possible all the elements which may be encountered in the usual habitat of the organism, water was taken from ponds, laboratory aquaria, etc., together with their animal and plant life. In other words, no definite method was used in selecting the material, but it was simply collected at random from what might be the abode of infusoria, and thoroughly boiled. Probably the only condition present in the life of this culture which could not be encountered by a wild *Paramecium* was that the water had been boiled, but this was essential in the experiments in order to obviate the possibility of the contamination of the culture by an active or encysted wild specimen. Conjugation was impossible in the direct lines of the culture on account of the frequent isolations and change of medium.

[1] Woodruff ('08[1]).

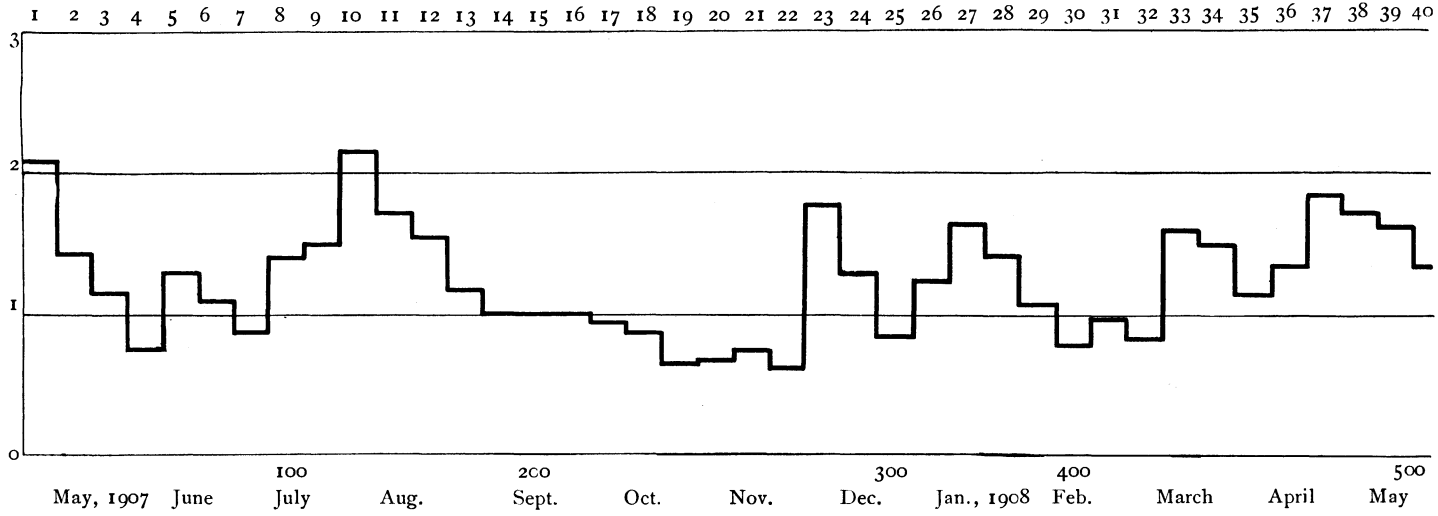


DIAGRAM I. PART I.

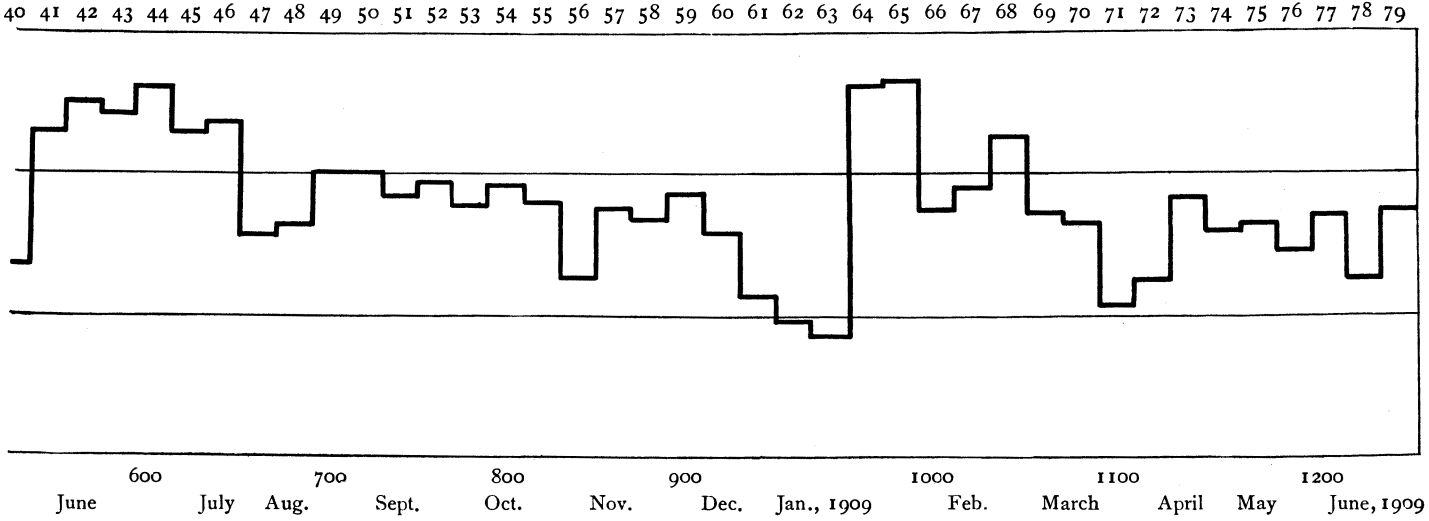


DIAGRAM I. PART 2.

FIG. 1. (Ten-day periods.) Complete history of *Paramecium aurelia* (*caudatum*), Culture I, from start on May 1, 1907, to the present time, June 29, 1909, at the 1,238th generation. The rate of division is averaged for ten-day periods.. The ordinates represent the average daily rate of division of the four lines of the culture. Above, the numbers of the ten day periods are indicated. Below, are designated the periods in which the fifteenth day of the respective months fell. The figures, 100, 200, etc., represent generations and are placed in the periods in which they were attained.

III. Description of Cultures.

Culture I has attained, during the first twenty-six months of its life, the 1,238th generation. The average rate of reproduction for the entire period has been over one and a half divisions per day, and during not a single ten-day period has the average rate fallen as low as one division in two days, while during several ten-day periods the rate has averaged over two and a half divisions per day.

Fig. 1 shows, by the familiar block method, the average rate of division of Culture I for ten-day periods. Especial emphasis is put on the character of this curve as the results of Calkins' cultures are plotted for ten-day periods. A study of this diagram shows that the life history falls naturally into two parts. The first extends from period one to period thirty, and the second extends from period thirty to the present time. These two major parts of the curve are coextensive with different methods in the treatment of the culture. The culture medium used during the periods covered by part one was very much more uniform than that used during part two; the decidedly varied environment not being employed until February, 1908. The effect of this treatment is shown in the decided change in the character of the curve of the division rate from that period to the present. The general vitality of the protoplasm is considerably higher as is shown by the fact that only once since that time has the curve for a ten-day period fallen below one division per day, and that period represents the culmination of the more or less severe treatment which the culture received when it was carried to Baltimore during convocation week. However, to the change of water and other conditions incident to the journey is apparently to be attributed the stimulus which enabled the culture to attain a short time after an average rate of nearly two and three quarters divisions per day (see Fig. 1, periods 64 and 65), the highest reproductive power shown in the life history for any ten-day period.

A study of Fig. 2, which is plotted by the same method as Fig. 1 except that the periods are of thirty-day instead of ten-day duration, also shows clearly the effect of the varied culture medium which has been in use from February, 1908. The rate

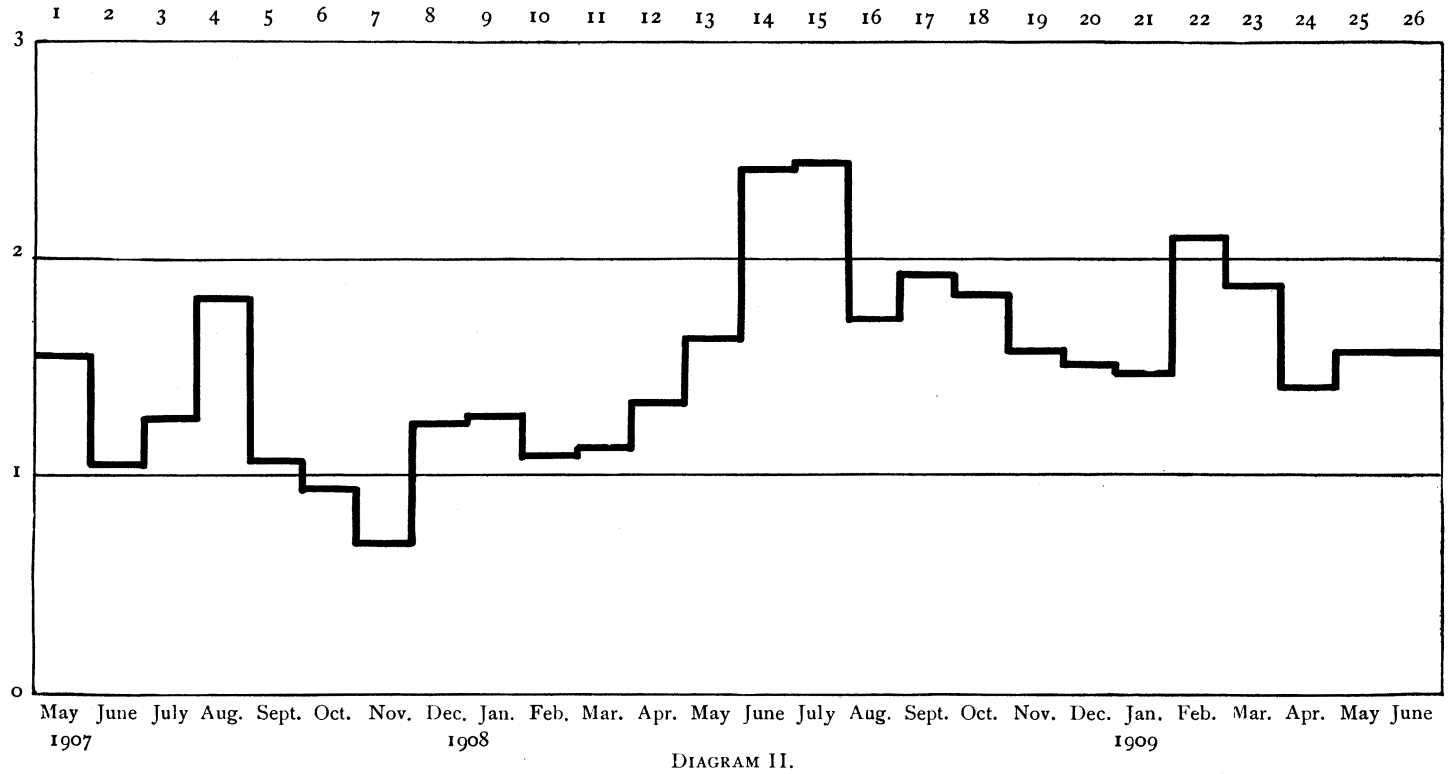


DIAGRAM II.

FIG. 2. (Thirty-day periods.) Complete history of *Paramecium aurelia* (*caudatum*), Culture I, from start on May 1, 1907, to the present time, June 29, 1909, at the 1,238th generation. The rate of division is averaged for each month of the life of the culture. The ordinates represent the average daily rate of division of the four lines of the culture.

of reproduction has never again fallen as low as it was when this treatment was begun. When averaged for thirty-day periods the highest rate of division appears in June and July, 1908; the great rise in vitality which occurred in February, 1909, and is shown in periods 64 and 65 of Fig. 1 (ten-day periods) is not so conspicuous, as the average is reduced by the low rate of fission during the two periods preceding.

A similar examination of Fig. 3, in which the rate of division is averaged for five-day periods, is not so instructive because the influence of the rhythms is more clearly brought out during short periods, so that the general trend of the curve of the life history is somewhat obscured. However, when the curve is surveyed in its entirety it illustrates the fact that the vitality of the organisms, as indicated by the fission rate, has maintained a higher average since the use of a promiscuous culture medium was instituted.

In order to determine more fully the effect of a very constant environment on this same race of *Paramecium* which was being maintained on a varied environment, there was isolated from each of the four lines, on February 19, 1909, at the 1,121st generation, a second culture, designated *Paramecium* I^s. This culture was submitted to as constant an environment as was practicable, according to the general method of Calkins. There were, then, two cultures of the same protoplasm running simultaneously, one being subjected to a varied or promiscuous culture medium, and the other to a comparatively constant culture medium. As a matter of precaution, and to show if there was anything intrinsically deleterious in the medium provided for Culture I^s, its constant medium was employed at various times as a temporary medium for Culture I. This, of course, simply increased the variability of the medium of Culture I. Also, near the end of the I^s series, its medium was employed not only for Culture I, but also for two cultures of ex-conjugant paramecia (*Paramecium* II^y and *Paramecium* II^z) from an entirely different source from that of the *Paramecium* of Culture I.

The results of these experiments with a constant and varied environment on the same protozoan protoplasm are shown graphically in Fig. 4. A glance at this curve shows that the vitality of the protoplasm of Culture I^s (constant environment), as meas-

ured by the fission rate, immediately fell below that of Culture I (varied environment), and that a consistent decrease in division rate was maintained until Culture I^s died out on June 6, 1909, at the 1,159th generation, after having been one hundred and seven days, or a little more than three months, on the constant medium; whereas the protoplasm of Culture I maintained about the same general average vitality throughout the period and had attained the 1,200th generation, a gain of forty-one generations in 107 days over the I^s culture. That the death of the I^s culture was not due to some sudden and accidental inimical change in the medium is proved by the fact that the same culture medium when used temporarily for the other cultures produced no deleterious effect, and also by the character of the curve of the fission rate of the I^s culture which has a consistent general downward trend except as it is affected by the rhythms. A comparison of the I^s culture curve and the curves of Calkins' *Paramecium* cultures shows a striking similarity in character. The cycles in Calkins' A culture were of six months duration and varied between 126 and 200 generations in length. My I^s culture passed through only 138 generations, but as it actually represents only the downward slope, or second half, of a cycle of Calkins' culture, my I^s cycle is really somewhat longer than those of Calkins. This point is only of interest in that it indicates in a general way the comparative similarity of the reactions of the protoplasm of paramecia from widely different sources to the same general conditions; and because it removes the possible objection that the I^s culture died out because it had been acclimated to the varied environment, and consequently it could not withstand the change to a constant medium. Of course, this is only a formal objection at best as there is every reason for supposing that the wild paramecia with which all cultures are started have been subjected for countless generations to considerably greater variations in their environment than it is possible to supply artifically.

IV. Discussion.

Up to the present time Culture I has not completed a "cycle" and all the fluctuations in vitality, as indicated by the division rate, fall under the head of "rhythms," as previously defined by me,[1]

[1] Woodruff ('05).

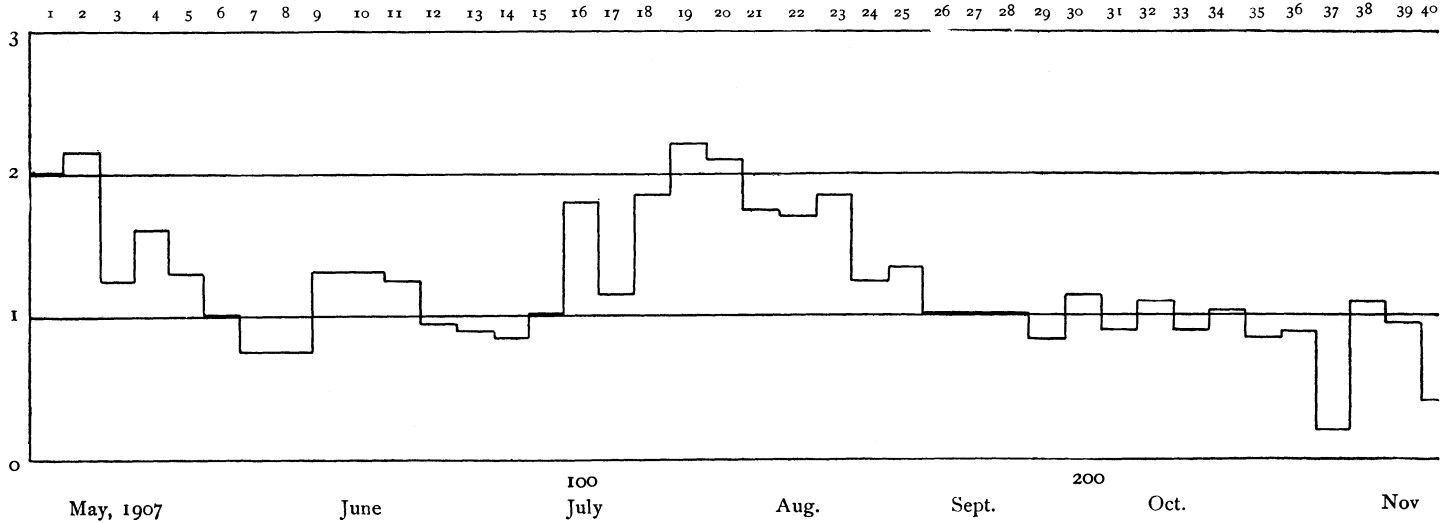
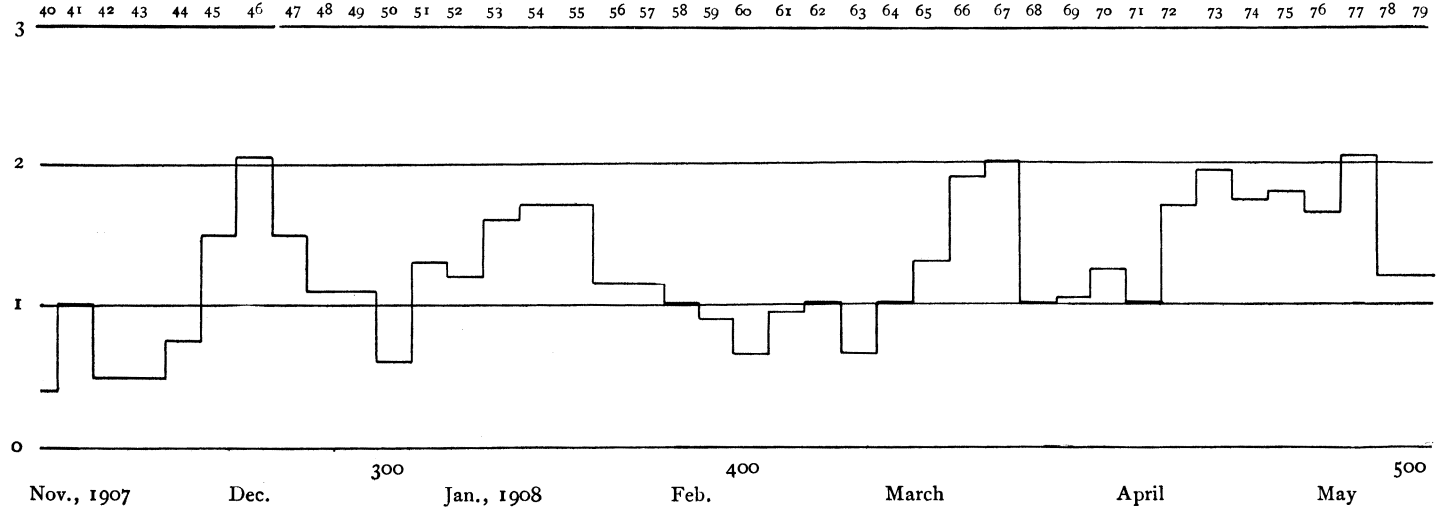


DIAGRAM III. PART 1.



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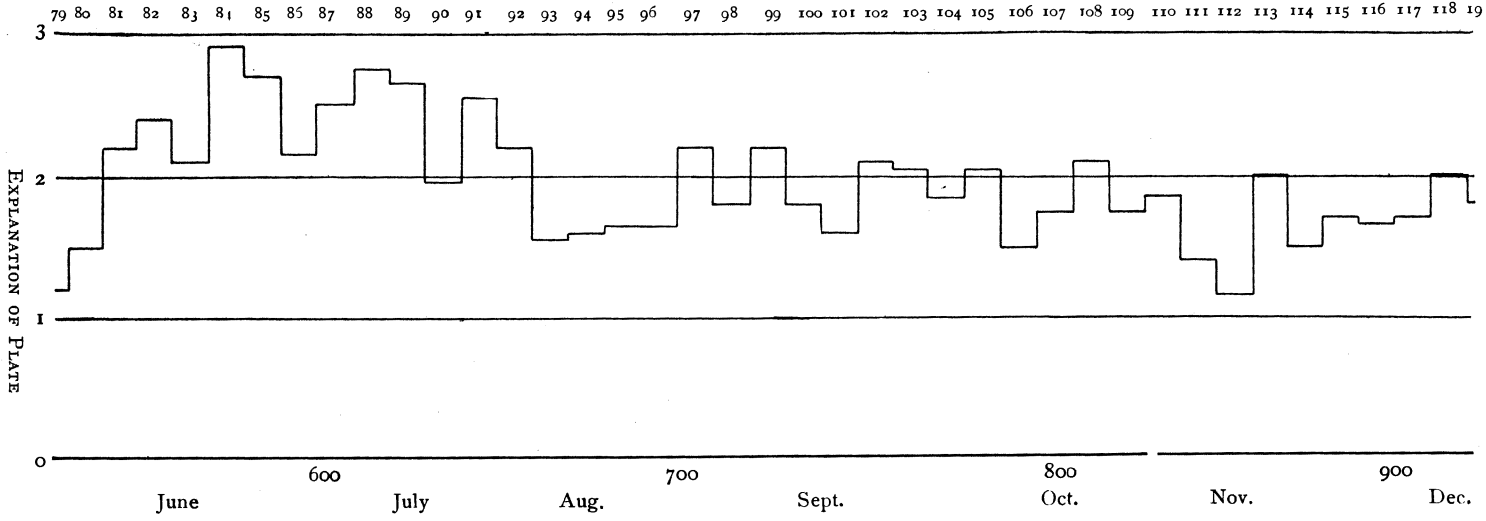

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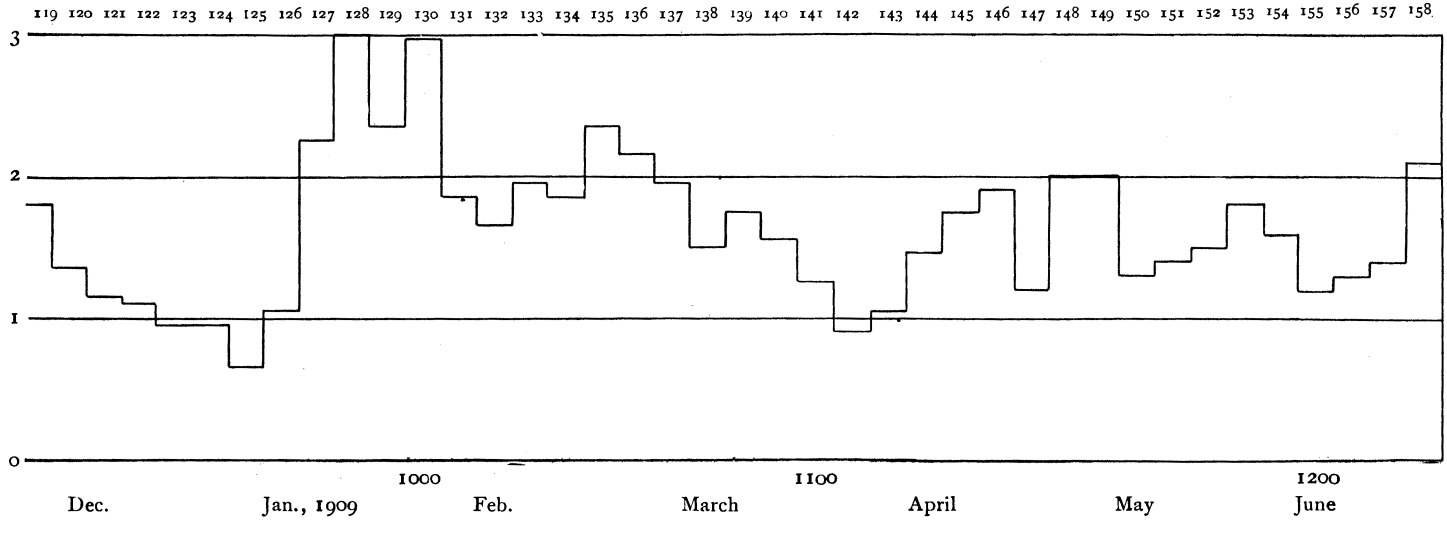

DIAGRAM III. PART 4.

FIG. 3. (Five-day periods.) Complete history of *Paramecium aurelia* (*caudatum*), Culture I, from start on May 1, 1907, to the present time, June 29, 1909, at the 1,238th generation. The rate of division is averaged for five-day periods. The ordinates represent the average daily rate of division of the four lines of the culture. Above, the numbers of the five-day periods are indicated. Below, are designated the periods in which the fifteenth day of the respective months fell. The figures, 100, 200, etc., represent generations, and are placed in the periods in which they were attained.

viz., "A rhythm is a minor periodic rise and fall of the fission rate, due to some unknown factor in cell metabolism, from which recovery is autonomous." The rhythms are more evident when a more constant environment is maintained, as I have shown in a study of the effect of a particularly stable environment on *Gastrostyla steinii*, during the months of July, August and September (cf. Fig. 5).

Gregory ('09) has plotted the curve of a *Stylonychia* culture for five-day periods from the data of Popoff ('07), which shows that the first four of the so-called "deep depression" periods emphasized by Popoff resolve themselves into "normal rhythms from which recovery is autonomous." Gregory also points out in her own 548 generation culture of *Tillina magna* that "the curve which represents the general vitality of the protoplasm shows the normal rhythmic fluctuations observed by Woodruff."

I have previously interpreted as rhythms the tri-monthly depressions in vitality, which Calkins and the earlier workers on *Paramecium* have noted, and the results obtained from my culture of *Paramecium* seem to indicate that the semi-annual cycles of Calkins are also actually rhythms, recovery from which was not autonomous under the conditions of a constant environment. The general occurrence of rhythms in the life history of infusoria is established, I believe, but to what they are due is still awaiting discovery.

Gregory has emphasized the point that "Enough consideration has not been taken of the fact that not only does each individual vary in its degree of sensitiveness at different periods in the life history, as suggested by Towle and shown by the rhythms of Woodruff, but each individual of the same species as well as of different species has its own peculiar protoplasmic reactions. Woodruff himself has failed to consider this fact in his last paper on the effects of a varied environment on *Paramecium*. . . . He cannot logically compare his results with those of Calkins for he is not dealing with the same protoplasm. . . ." In 1905 I wrote: "My cultures lead me to believe, with Simpson, that the personal equation, if I may use that term, of the individual selected to start a culture has the most influence in determining the number of generations attained. . . . Calkins' discovery of

what he calls 'incipient fertilization' . . . would seem to bear out this point, and to show that the number of generations, or the period, over which a cycle extends, is not a point of great moment."

I have since found no reason to alter this opinion. But there must be *limits* beyond which the "peculiar protoplasmic reactions" of any individual do not extend, otherwise each would be a law unto itself and there would be as many laws as individuals. Certainly we may reasonably assume that there are limits of time, and generations, which a "cycle" (if it exists) of any particular species will not exceed. The earlier investigations apparently indicated that about three months or about 100 generations was the limit of the cycle of *Paramecium.* Calkins in his last paper extended the cycle to about six months, or about 200 generations. The present culture extends the "cycle" to more than twenty-six months, and more than one thousand two hundred generations. The longest culture carried by Calkins (Culture A) lived for twenty-three months, and attained 742 generations — but this comprised four complete cycles, the last one terminating fatally. It is necessary to contrast the cycle of Calkins' culture of about six months duration, and two hundred generations, with the life (cycle) of this culture, which is of twenty-six months duration at present, and 1,238 generations. That is, this culture shows a "cycle" twenty months longer in time, and, so far, of over one thousand more generations.

The *character* of the life history must also be taken into account. There is a marked difference in the character of the *Paramecium* curve after February, 1908, when the decidedly varied environment was begun (cf. Figs. 1 and 2). A similar difference in character is evident in the *Gastrostyla* culture when the more constant medium was being maintained (cf. Fig. 5, July, August and September), and the same is again strikingly shown in the present culture of *Paramecium* in the experiments which subjected the "same protoplasm" to a constant and a varied environment simultaneously (cf. Fig. 4).

The term cycle, as has been pointed out, is a relative one, but I think it is necessary to extend the conception of the cycle (as worked out on infusoria on constant media) to an unwarranted

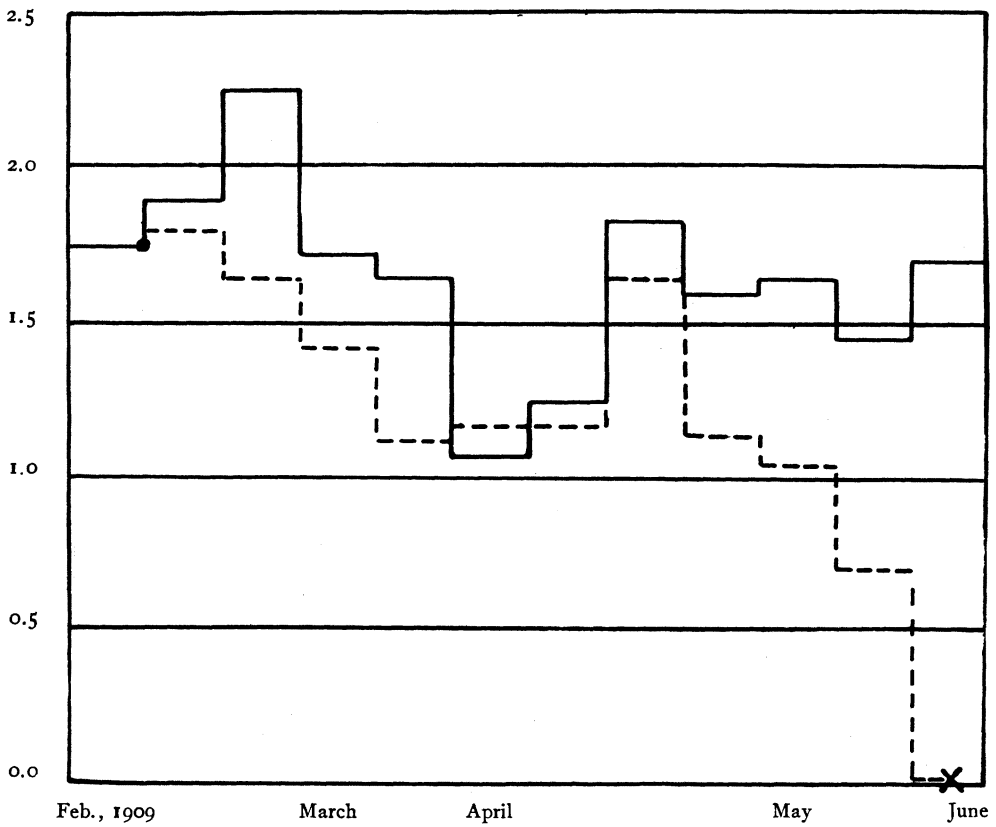

DIAGRAM IV.

FIG. 4. *Paramecium*, Culture I^s (constant environment) = broken line; Culture I (varied environment) — continuous line. ● = point of isolation of Culture I^s from Culture I. *X* = point at which Culture I^s died out. Other details as in Fig. 1.

extent if it is to be made to include the life history of the present culture. I would not suggest that the protoplasm of every wild *Paramecium* has the potential to attain over twelve hundred generations or more — undoubtedly there are strong and weak strains among infusoria as among other classes of animals. Again, it is possible that the different races of *Paramecium* which Jennings ('08) has been able to isolate may have a physiological as well as a morphological basis of distinction. It may be also that I have been particularly fortunate in my haphazard selection of culture material, so that the proper variations have been available when necessary. It is true that any particular ingredient of the infusion which might be needed would not long be available for the organisms on account of the frequent change of the character of the infusion, and Gregory has recently shown in the case of *Tillina*, and I have shown in the case of *Gastrostyla*, that daily stimulation with salts is often more efficacious than an initial stimulation. But Calkins says in regard to the second cycle of his A culture ". . . in December it was necessary to keep them on the stimulant only a day or two to get the desired result. The short treatment at this period sufficed, because they were not allowed to become weakened to the same extent as in the preceding period of depression." It is this factor which has been taken into account in this study, and it is probable that it has contributed largely to the vitality of the culture.

It must also be kept in mind that a certain amount of judgment is exercised in selecting a representative specimen for isolation. By experience one becomes quite familiar with the normal movements, shape, and general appearance of the organisms, so that it is possible to select a favorable specimen daily for the continuance of each of the lines. The precaution is nearly always taken to examine the culture again a few hours after the isolations to see how the organisms behave in the fresh culture liquid. If everything does not appear normal, a new set of individuals is isolated from the "stock" (*i. e.*, from the one, three or seven individuals left after isolation, the number depending on the rate of division during the previous twenty-four hours). Undoubtedly the process practically results in the artificial selection of the organisms which have the highest poten-

tial of division and those which are most readily acclimated to changes in their medium. Each and all of these factors may contribute to the length of the life of the culture — but after all is done the "chances" are largely against the prolonged life of the culture.

This culture suggests, then, the time-honored question whether the protoplasm of infusoria has the potential of unlimited life and reproduction, and the fundamental question as to the rôle of conjugation in the life history of these organisms. Up to the present time there has been no tendency to conjugate among the individuals of this culture, although in the "stock" cultures, consisting of the individuals remaining over after the daily isolations, there has been ample opportunity for it to take place. The daily isolations, of course, have precluded its occurrence in the four direct lines of the culture. This result agrees with those of Joukowsky on a 460 generation culture of *Pleurotricha lanceolata*, Gregory on a 548 generation culture of *Tillina magna*, and Woodruff on an 860 generation culture of *Oxytricha fallax*, on a 448 generation culture of *Pleurotricha lanceolata* and on a 288 generation culture of *Gastrostyla steinii*. Maupas secured no conjugations in his cultures of *Stylonychia mytilus* and *Oxytricha* sp., though his other series yielded plenty of syzygies. That the infusoria do conjugate is, of course, a matter of common observation; but I believe these results indicate that the phenomenon is not so frequent in the life history as is generally believed. A daily examination of twenty hay infusions, made up by several different methods, has not shown a single case of conjugation among the hypotrichous forms present either at the top or bottom of the jars. In fact, not a single syzygy has been observed in any species except *Paramecium*, and in this form conjugation has been very rare. However, a sudden transference of the paramecia from the comparatively constant culture medium of a hay infusion to a different medium has produced marked epidemics of conjugation. It is just possible that a constant medium is necessary for the so-called miscible state (Calkins) to develop, and that this becomes functional on transference to a decidedly different medium. If this is so, it may account for the absence of conjugation in my paramecia series on a varied medium, and

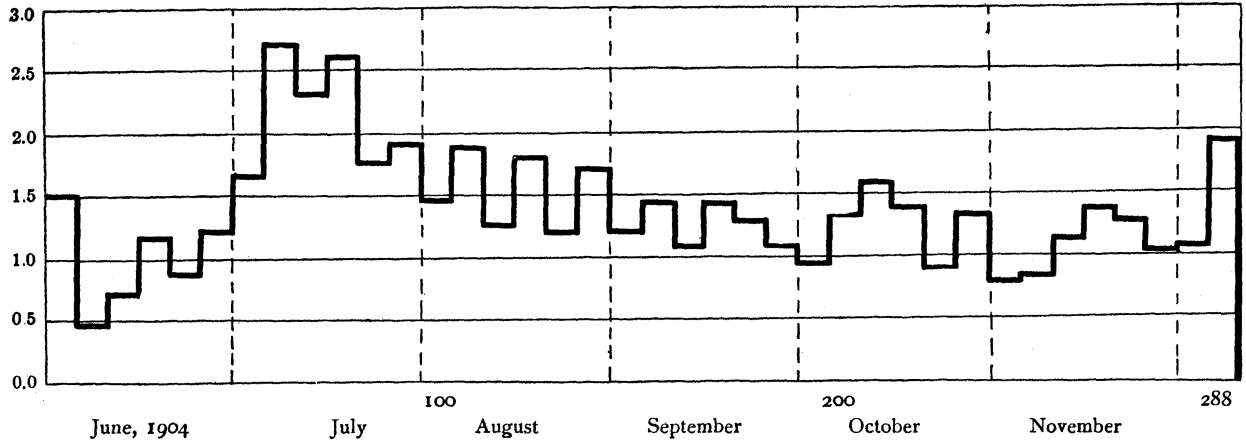

DIAGRAM V.

FIG. 5. Complete history of *Gastrostyla steinii*, Culture A, averaged for five-day periods. Method of plotting is the same as in previous diagrams.

its prevalence in Calkins' cultures on a constant medium. This idea is not supported by my hypotrichous cultures which were carried on a constant medium and still did not develop a tendency to conjugate even when the culture medium was varied in some special experiments. It is highly probable, however, that the superficial conditions which induce conjugation may vary in different species.

Periods of marked physiological depression have not appeared during the first twenty-six months of the life of the *Paramecium* I culture, but well-defined morphological changes have taken place. I shall not discuss these cytological changes at present, as I believe it is advisable to wait until the culture is terminated naturally, or by accident, so that all the data from the complete series may be discussed in its entirety. It is clear, however, that the relation of the rate of division to the so-called "normal" condition of the nuclei of *Paramecium* is not supported by this culture, as decided nuclear changes apparently do not affect the general vitality of the organisms. It may be noted further, that not a single monster due to incomplete or otherwise abnormal division has occurred in the entire 1,238 generations.

V. Conclusions.

The experimental study of the life history of infusoria has so far clearly shown that:

The protoplasm of these organisms, when subjected to a comparatively constant culture medium, passes through long cyclical changes in vitality which finally result in the death of the organism.

The protoplasm may be "rejuvenated" by suitable changes in the culture medium (stimuli) at critical points in the cycle, and thus be enabled to resume active reproduction for a longer period.

The essential fact brought out by this study is that:

The protoplasm of the individual *Paramecium* isolated over two years ago to start the culture has had the potential to divide (so far) over one thousand two hundred and thirty times at an average rate of more than three divisions every two days, and the representatives of the untold millions of its progeny which are

still in captivity give every indication of being in as normal physiological and morphological condition as their ancestor. This suggests that when the protoplasm is constantly subjected to a suitable varied environment the cycle may be greatly prolonged and probably entirely eliminated — the fluctuations in vitality not transcending the rhythm.

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